Variation in Sperm Head Morphology of Muroid Rodents of Africa: Phylogenetic Implications

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ABSTRACT

Sperm morphology from individuals of the following subfamilies of muroid rodents of southern Africa is determined: Cricetomyinae (*Saccostomus*), Gerbillinae (*Gerbillurus* and *Tatera*), Dendromurinae (*Dendromus*, *Malacothrix*, *Prionomys*, *Steatomys*, and *Deomys*), and Otomyinae (*Otomys*); *Mystromys* (subfamily Mystromyinae) is used as an outgroup. The sperm head of most species including that of *Mystromys* is falciform in shape but differences in internal organisation occur. In *Mystromys* it is long and thin and there is a very large apical acrosomal segment. The falciform sperm heads of *Saccostomus* and *Malacothrix* are broader basally but have a similar organisation apically, whereas in *Deomys* the sperm head is very small, bilaterally flattened, with no apical hook. *Gerbillurus* sperm head terminates in a sharp pointed apex, whereas in *Tatera* it is round apically and a deep invagination occurs in the caudal nuclear region. In *Otomys* the sperm head is falciform and the organisation of the perforatorium and acrosome is similar to that of most murine rodents apart from *Acomys* and *Uranomys*. This study suggests that a falciform sperm type is probably the ancestral condition for the dendromurine-cricetomyine-otomyine-murine clade. Sperm of both *Deomys* and *Tatera* are highly divergent, and those of the otomyines and murines are very different from sperm of species in other subfamilies; since a very similar or identical morphology occurs in most species of the Otomyinae and Murinae it suggests that these two subfamilies are sister groups to the exclusion of the other subfamilies and of *Acomys* and *Uranomys*.

RÉSUMÉ

Les variations morphologiques de la tête des spermatozoïdes des Rongeurs Muroïdes d'Afrique: implications phylogénétiques

La morphologie du spermatozoïde a été déterminée chez des spécimens appartenant aux familles suivantes de Rongeurs Muroïdes d'Afrique du Sud : Cricetomyinae (*Saccostomus*), Gerbillinae (*Gerbillurus* et *Tatera*), Dendromurinae (*Dendromus*, *Malacothrix*, *Prionomys*, *Steatomys* et *Deomys*), et Otomyinae (*Otomys*). *Mystromys* (sous-famille Mystromyinae) a été utilisé comme outgroup. La tête du spermatozoïde de la plupart des espèces, y compris celle de *Mystromys*, est falciforme, mais il existe des différences dans l'organisation interne. Chez *Mystromys* la tête est longue et fine et un grand segment acrosomien apical est présent. Les têtes falciformes de *Saccostomus* et *Malacothrix* sont plus larges à la base mais ont une même organisation à l'apex, alors que la tête de *Deomys* est très petite, aplatie bilatéralement, et sans crochet. La tête de *Gerbillurus* se termine en un apex très effilé, alors que chez *Tatera* la tête est ronde à l'apex et une invagination profonde existe dans la région nucléaire caudale. Chez *Otomys* la tête est falciforme et l'organisation du perforatorium et de l'acrosome est similaire à celle de la plupart des Rongeurs Muridae, à part *Acomys* et *Uranomys*. Cette étude suggère que le spermatozoïde de type falciforme est la condition ancestrale pour le clade Dendromurinae-Cricetomyinae-Otomyinae-Murinae. Les spermatozoïdes de *Deomys* et de *Tatera* sont très divergents, et ceux des Otomyinae et Murinae sont très différents de ceux des espèces des autres sous-familles. Comme cette morphologie très similaire ou identique se trouve chez la plupart des espèces des Otomyinae et Murinae, cela suggère que ces deux sous-familles sont des groupes-frères, à l'exclusion des autres sous-familles et d'*Acomys* et *Uranomys*.

BREED, W. G., 1995. — Variation in sperm head morphology of muroid rodents of Africa: phylogenetic implications. *In*: JAMIESON, B. G. M., AUSIO, J., & JUSTINE, J.-L. (eds), Advances in Spermatozoal Phylogeny and Taxonomy. *Mém. Mus. natn. Hist. nat.*, **166** : 409-420. Paris ISBN : 2-85653-225-X.

The superfamily of muroid rodents is composed of over 1300 species of about 280 genera with major radiations in both the New and Old Worlds. SIMPSON [37] proposed two major families: the Cricetidae and the Muridae with the latter including the African climbing mice (Dendromurinae), swamp rats (Otomyinae), as well as true rats and mice of the Old World (Murinae) inclusive of the African pouched mouse *Saccostomus*. Subsequently it was suggested that the Dendromurinae [26] and Otomyinae [28] were closer to the Cricetidae, and that *Saccostomus* and other cricetomyines were also part of this family [31, 32]. CHALINE *et al.* [14] later erected several new families: the Dendromuridae, Cricetomyidae, Nesomyidae (which included the otomyines), whereas REIG [34] proposed these groups as subfamilies within the Cricetidae. CARLETON & MUSSER [12] expanded the family Muridae to embrace 15 subfamilies including the Dendromurinae, Otomyinae, Murinae, Gerbillinae, Cricetinae and others. Over the last 10 years palaeontological and biochemical data have suggested that the dendromurines, cricetomyines, and gerbillines are probably more closely related to each other than any is to the Cricetinae [11, 13, 18].

Apart from the unstable higher order taxonomy, it has also become apparent that at least one genus placed within the Murinae, *Acomys*, may not be part of this group [36, 40]. Recent DNA-DNA hybridisation studies have suggested that it, together with *Uranomys* and *Lophuromys*, form a monophyletic clade that clusters with the Gerbillinae [15, 30], whereas dental features of *Acomys* and *Uranomys* appear to be intermediate between this group and the Murinae [16].

Since the taxonomy and phylogenetic relationships between these major groups of African muroids is controversial, it would seem timely to explore the use of other data sets for hypothesising relationships. Over the last few years spermatozoal morphology has been used with varying degrees of success as an independent character for suggesting relationships between various marsupials [22, 24, 38], bats [23], American cricetids [27], and Asian and Australasian murids [5, 7, 8, 10]. Here it is used to explore relationships between the subfamilies of African murids and, in particular, between members of the Murinae, Dendromyinae, Gerbillinae, Cricetomyinae and Otomyinae subfamilies. As an outgroup comparison, the sperm morphology of *Mystromys* (an archaic African species usually placed in the Mystromyinae) is considered. To date there have been only a few investigations on morphology of spermatozoa of African murid rodents [e.g. 1, 3, 6, 21, 39], and there appear to be no studies using it to determine the relationships between the murid subfamilies. This is the aim of the present study.

MATERIAL AND METHODS

Sperm morphology was investigated by light (LM) and, where possible, transmission electron microscopy (TEM) from: *Mystromys alba* (subfamily Mystromyinae), *Prionomys batesi*, *Steatomys parvus*, *Dendromus mystacalis*, *D. mesomelas*, *Deomys ferrugineus*, and *Malacothrix typica* (subfamily Dendromurinae), *Saccostomus campestris* (subfamily Cricetomyinae), *Otomys irroratus* (subfamily Otomyinae), and *Gerbillurus paeba* and *Tatera leucogaster* (subfamily Gerbillinae). The sources of animals were: *M. alba* and *S. campestris* from the South African Institute for Medical Research, Sandringham, Johannesburg, one *O. irroratus* from near Pietermaritzburg and another from Ngare Forest, Natal Province. *G. paeba* came from near Swakopmond, and a *T. leucogaster* from Etosha pan, Namibia. A second *T. leucogaster* was collected in northern Quazulu. The *M. typica* specimen came from near Hutchinson, Cape Province (Transvaal Museum No 36676), and material from the other dendromurines was from specimens at the Natural History Museum, London which included *D. ferrugineus* from Kivu Province, Zaire, *D. mystacalis* from Babaka, Isai River, Huri, Zaire, *D. mesomelas* from Uganda, and *S. parvus* from Swaziland.

Caudae epididymides were immersed in either 10% phosphate buffered formaldehyde or 3% paraformaldehyde/3% glutaraldehyde made up in 0.1 M phosphate buffer, pH 7.4. For LM, spermatozoa were extruded from the ducts and placed on microscope slides. For TEM the tissue was rinsed in phosphate buffer, fixed in osmium tetroxide, rinsed again, dehydrated by passing through a graded ethanol series, and embedded in Araldite. Thick and thin plastic sections were cut, the latter stained with lead citrate and uranyl nitrate, and observed with a JEOL 100S and/or Philips CM 100 EM.

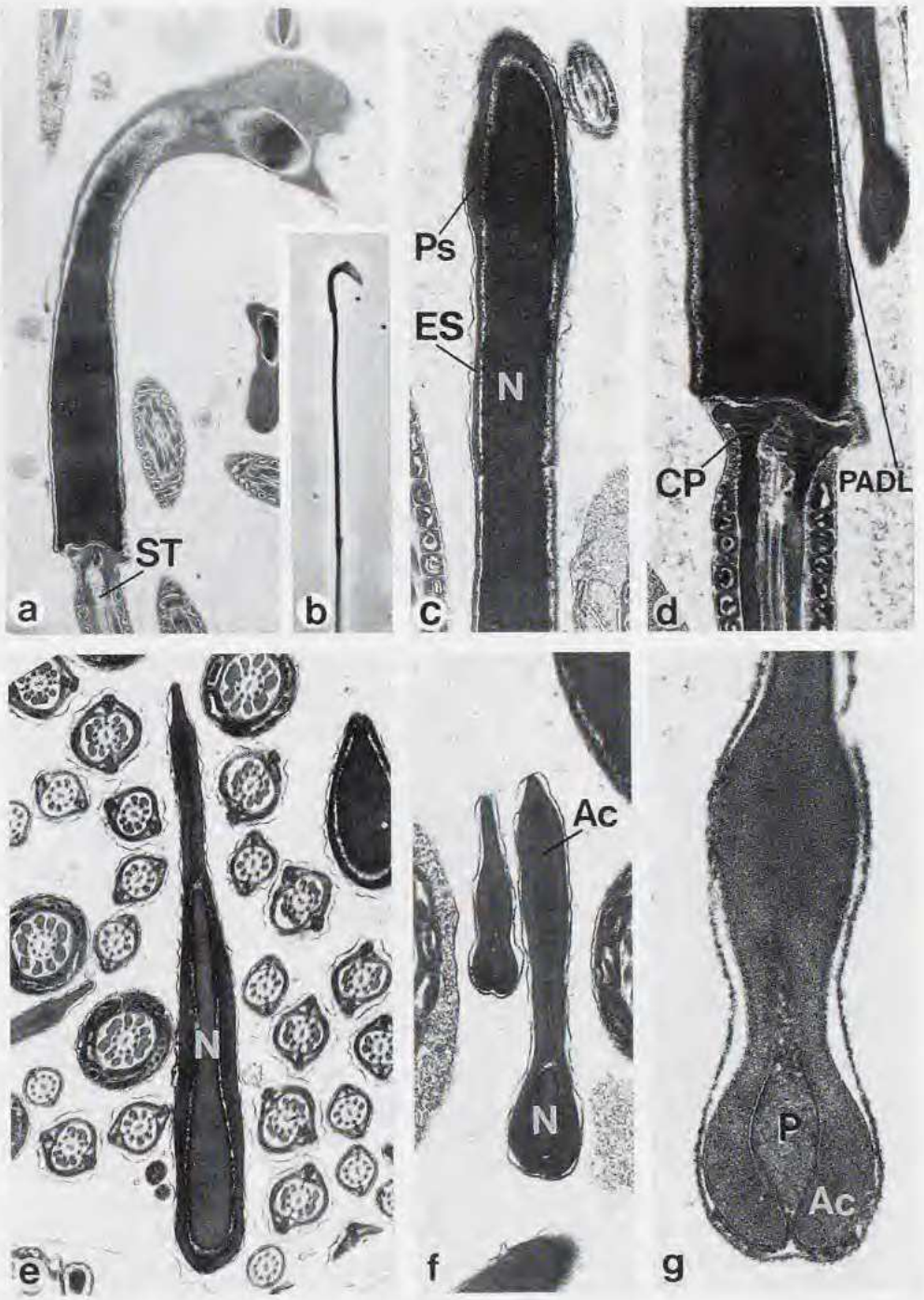


FIG. 1. — **a-g:** Sperm of *Mystromys alba*. **a, b:** Head is falciform. **c:** An electron-dense nucleus (N) is capped by a large acrosome which has a narrow equatorial (ES) and wider principal segment (PS); **d:** a postacrosomal dense lamina (PADL) occurs in the posterior region of sperm head; **e, f:** anterior region of nucleus is bilaterally flattened and capped by a massive apical acrosome segment; **g:** TS of rostral tip indicates perforatorium (P) surrounded by acrosome (Ac) except midventrally. a, x 8 200; b, x 900; c, x 19 300; d, x 20 100; e, x 17 100; f, x 26 800; g, x 97 000.

RESULTS

Terminology used for the planes of section follows that of LALLI & CLERMONT [25]; thus in a falciform sperm head the convex surface is referred to as dorsal and concave as ventral. The space between the inner acrosomal membrane and outer nuclear envelope is the subacrosomal space and, when extensive, the perforatorium. The taxonomy proposed by CARLETON & MUSSER [12] is, in general, used although the family name of Cricetidae [37] is also referred to.

Mystromyinae

Mystromys alba.The sperm head is falciform with a length of 8-9 µm, maximum breadth of only 1.5 µm, hook length of 5 µm, and connecting piece of tail attaches midbasally with midpiece length of 38 µm and principal and end piece of about 100 µm (Fig. 1a, b).

The bilaterally flattened head has a homogeneous, electron-dense, nucleus that passes into the apical hook (Fig. 1a) which is capped by an acrosome that has a thicker principal than equatorial segment caudal to which is a postacrosomal dense lamina (Fig. 1c, d).

In the anterior region of the sperm head there is a massive apical acrosomal segment that extends at least 1 µm beyond the convex nuclear margin. The acrosomal matrix is more electron-dense medially (Fig. 1e, f), and anterior to the convex nuclear surface, beneath the inner acrosomal membrane, a subacrosomal space with electron-dense material is present (Fig. 1f).

The acrosome and perforatorium extend beyond the rostral tip of the nucleus. Cross sections demonstrate that the acrosome is bilaterally flattened and surrounds the perforatorium, except mid ventrally (Fig. 1g); the perforatorium is spear-shaped in cross section and the anterior region of acrosomal segment widens slightly before tapering apically (Fig. 1g).

Gerbillinae

Gerbillurus paeba. Sagittal (Fig. 2a, b) and frontal (Fig. 2c) TEM sections of the sperm head show that it is broad basally and narrows apically. The connecting piece of the sperm tail attaches to an off-centre basal position. There is a large apical acrosomal segment with a narrower principal segment over the anterior part of the nucleus posterior to which the equatorial segment passes down over much of the convex margin of the nucleus with the postacrosomal dense lamina passing around the posterior caudal margin (Fig. 2a). On the concave surface the equatorial segment is restricted anteriorly (Fig. 2b). Frontal sections through the anterior region of the sperm head indicate an invagination of the inner acrosomal membrane within which electron-dense material of the subacrosomal space occurs (Fig. 2c).

Tatera leucogaster. The sperm morphology of this species is highly divergent. Longitudinal TEM sections indicate that sperm head is bilaterally flattened (Fig. 2d), and has a deep invagination posteriorly (Fig. 2d, e, f). The nucleus tapers apically and its anterior four-fifths is capped by a nearly symmetrical acrosome (Fig. 2d) most of which is composed of a principal segment with the equatorial segment forming a girdle around the posterior nuclear region. The postacrosomal dense lamina is very short. A modest subacrosomal space is present with a small apical extension (Fig. 2d). A complex structural organisation of the connecting piece occurs with the basal plate running parallel to the long axis of the sperm head, and laterally an extension of the nuclear envelope within which material that exhibits light and dark banding occurs (Fig. 2f). The capitulum of the connecting piece passes vertically up into the implantation fossa and narrows apically (Fig. 2f).

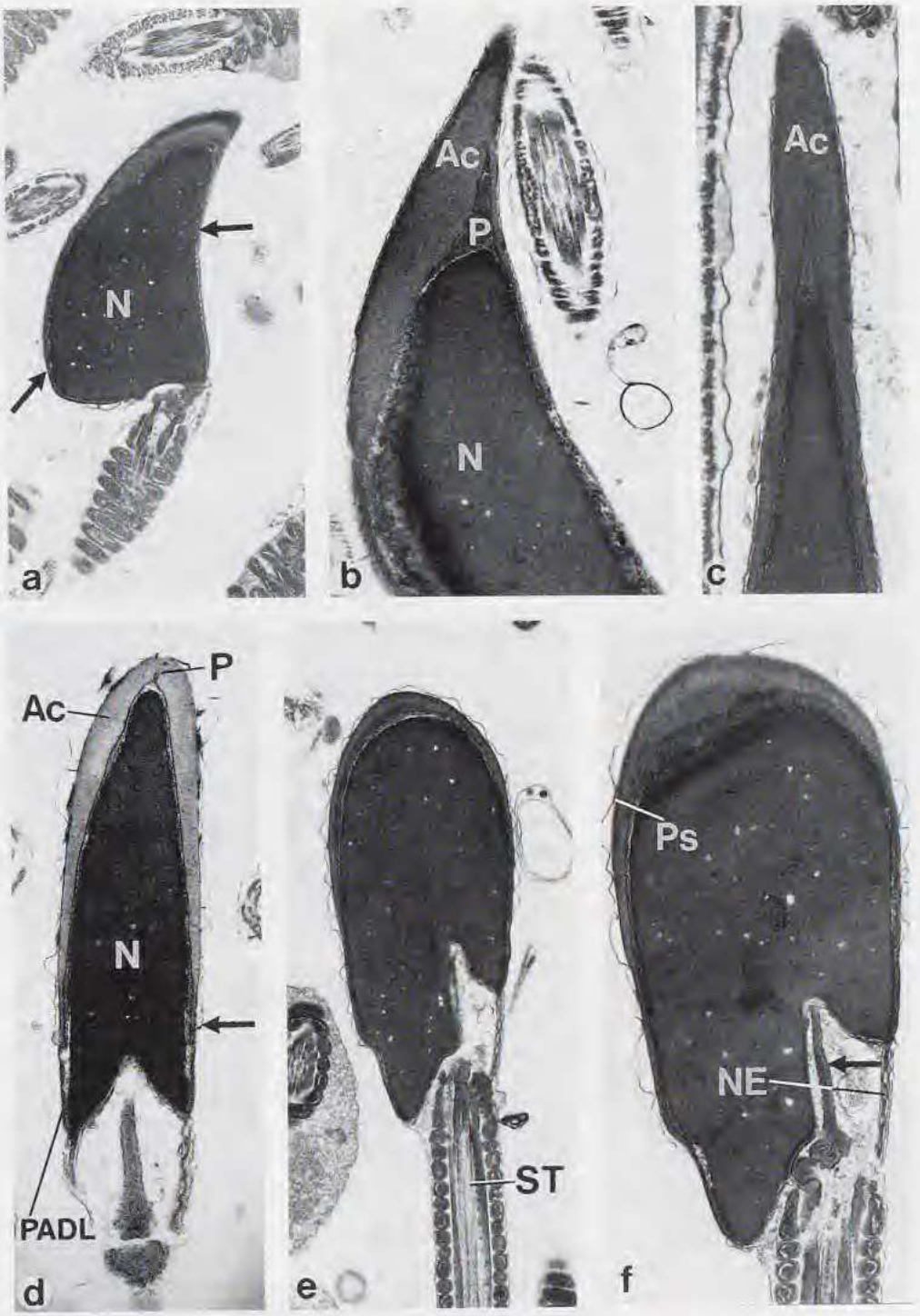


FIG. 2. — *Gerbillurus paeba* and *Tatera leucogaster*. **a-c:** Sperm of *G. paeba*. **a:** Sperm head narrows apically. **b, c:** anterior to the nucleus (N) there is a perforatorium (P) covered by part of the acrosome (Ac). **d-f:** *T. leucogaster*. **d:** spermatozoa have bilaterally flattened nuclei capped by a nearly symmetrical acrosome with small equatorial segment (arrow). **e, f:** connecting piece is unusual in having vertically projecting basal plate and partly bordered by fold of nuclear envelope (NE). a, x 9 100; b, x 15 000; c, x 31 600; d, x 18 200; e, x 11 200; f, x 17 400.

Cricetomyinae

Saccostomus campestris. The sperm head is falciform, has a length of 7 µm, maximum breadth basally of 3 µm, and apical hook, which curves sharply caudad, of about 6 µm (Fig. 3a). The sperm tail connects to the midbasal region, and has a midpiece length of about 25 µm and principal and end piece of about 100 µm.

The acrosome is largely restricted to the convex (Fig. 3b, c) and upper lateral surfaces of the sperm head with the posterior region forming a narrow equatorial segment (Fig. 3e, f). Anteriorly the inner acrosomal membrane is invaginated medially within which electron-dense material of the subacrosomal space occurs (Fig. 3f). Transverse sections of the apical hook show that basally it is bilaterally flattened and contains a nuclear extension which is completely surrounded by the acrosome except midventrally (Fig. 3g). Most of the hook does not contain nuclear material but the acrosome passes along its length and distally becomes triangular in cross section (Fig. 3h, i). In addition, within the apical hook, a small midventral extension of the subacrosomal space occurs which is in contact with the plasmalemma (Fig. 3h, i).

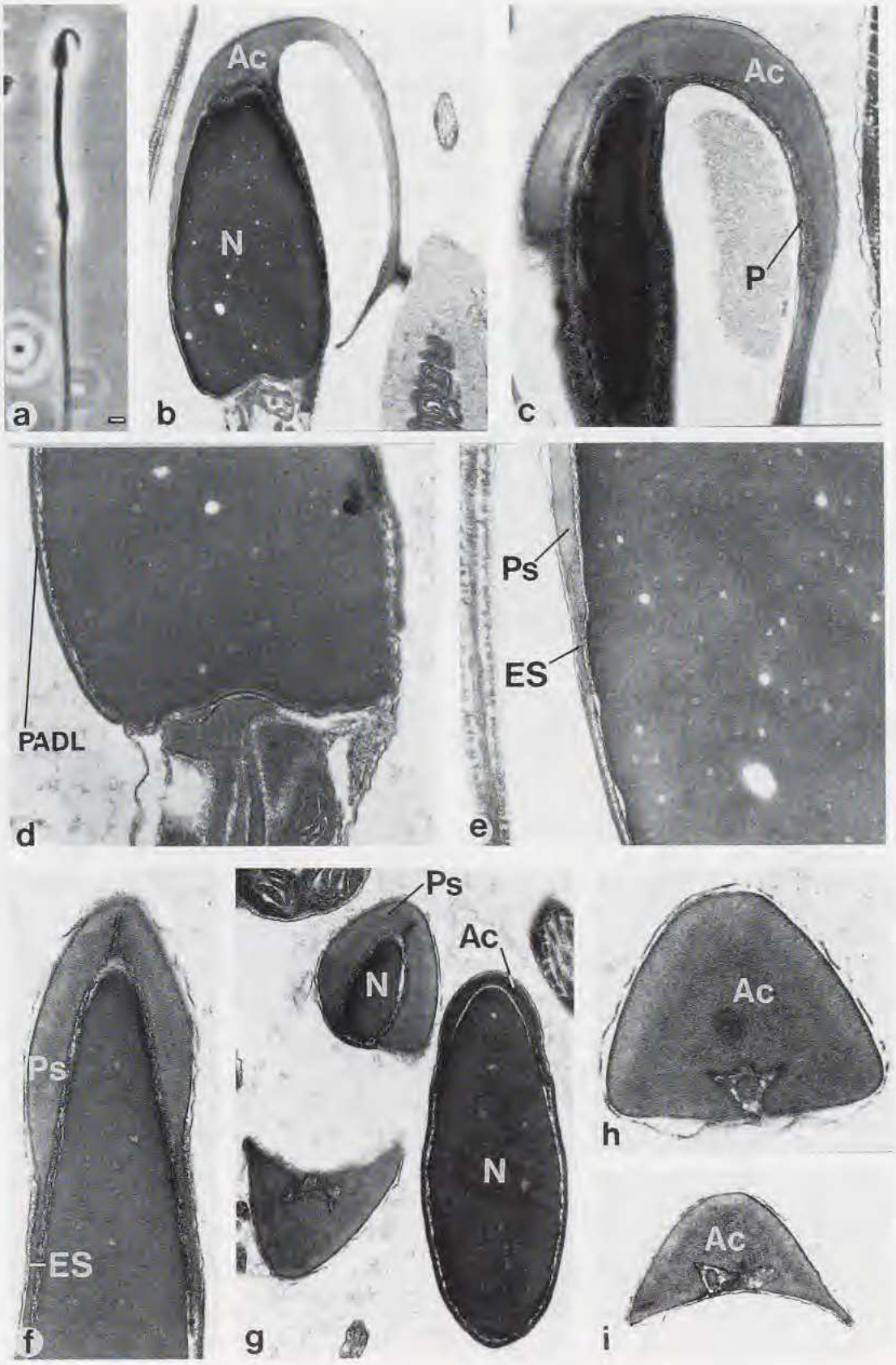
Dendromurinae

Prionomys batesi, *Steatomys parvus*, *Dendromus mesomelas*, *D. mystacalis*, *Malacothrix typica* and *Deomys ferrugineus*. Only material from *M. typica* was available for TEM. LM indicates significant interspecific differences in sperm head morphology. In *P. batesi* and *S. parvus* a small triangular sperm head is evident that is broadest basally and tapers apically to a hook that is flexed caudad. The connecting piece of the tail attaches midbasally (Fig. 4a, d). Sperm heads of *D. mystacalis* and *D. mesomelas* (Fig. 4b, c) are similar in form, have a lateral face of about 2 µm in diameter, and a 3-5 µm hook that extends from the convex surface and projects caudally, and on the ventral margin a small anteriorly-projecting spike. The sperm tail attaches off-centre basally and is about 90 µm long.

The third sperm type of *Deomys ferrugineus* (Fig. 4e) has a very small, 5 µm, bilaterally flattened head that lacks an apical hook. Anteriorly there appears to be a somewhat ridged, cap-like structure, presumably the acrosomal region, and posteriorly the sperm head tapers a little towards the connecting piece of the sperm tail which has a midpiece of 22 µm and principal and end piece of about 70 µm.

TEM of *Malacothrix typica* sperm shows that its head is largely composed of an electron-dense nucleus which does not appear to extend into the long apical hook (Fig. 4g, h). The acrosome is present along much of the convex surface and makes up most of the material in the apical hook which becomes triangular in cross section. There is a typical subacrosomal space between the inner acrosomal membrane and outer nuclear envelope that extends into the apical hook and is in contact with the plasmalemma midventrally.

FIG. 3. — Sperm of *Saccostomus campestris*. **a, b:** Sperm head is falciform with midbasal attachment of tail. **b, c:** apical hook is long and largely composed of acrosome (Ac). **d:** posterior to the acrosome is the postacrosomal dense lamina (PADL). **e:** acrosome has a very short equatorial segment (ES). **f:** frontal sections show perforatorium extends apically. **g:** TS through apical hook shows nucleus basally distal to which the hook becomes triangular and composed of acrosome except for small perforatorial extension. **h, i:** small perforatorial extension. a, x 900; b, x 11 400; c, x 17 000; d, x 26 600; e, x 33 000; f, x 35 800; g, x 22 700; h, x 50 100; i x 25 300.



Otomyinae

Otomys irroratus. Light microscopy indicates that the sperm head is falciform with the connecting piece of the sperm tail attached on the lower concave surface (Fig. 4f).

TEM shows that a typical bilaterally flattened, electron-dense, nucleus extends into the apical hook which is triangular in cross section. Within the hook the acrosome splits into a dorsal and dorsolateral crest over the convex region and a much smaller, flat, head cap segment close to the ventral margin. Most of the material in the apical hook is composed of the perforatorium that has three prongs, one that is close to the medial convex margin and the other two, much larger ones, which occur ventrolaterally.

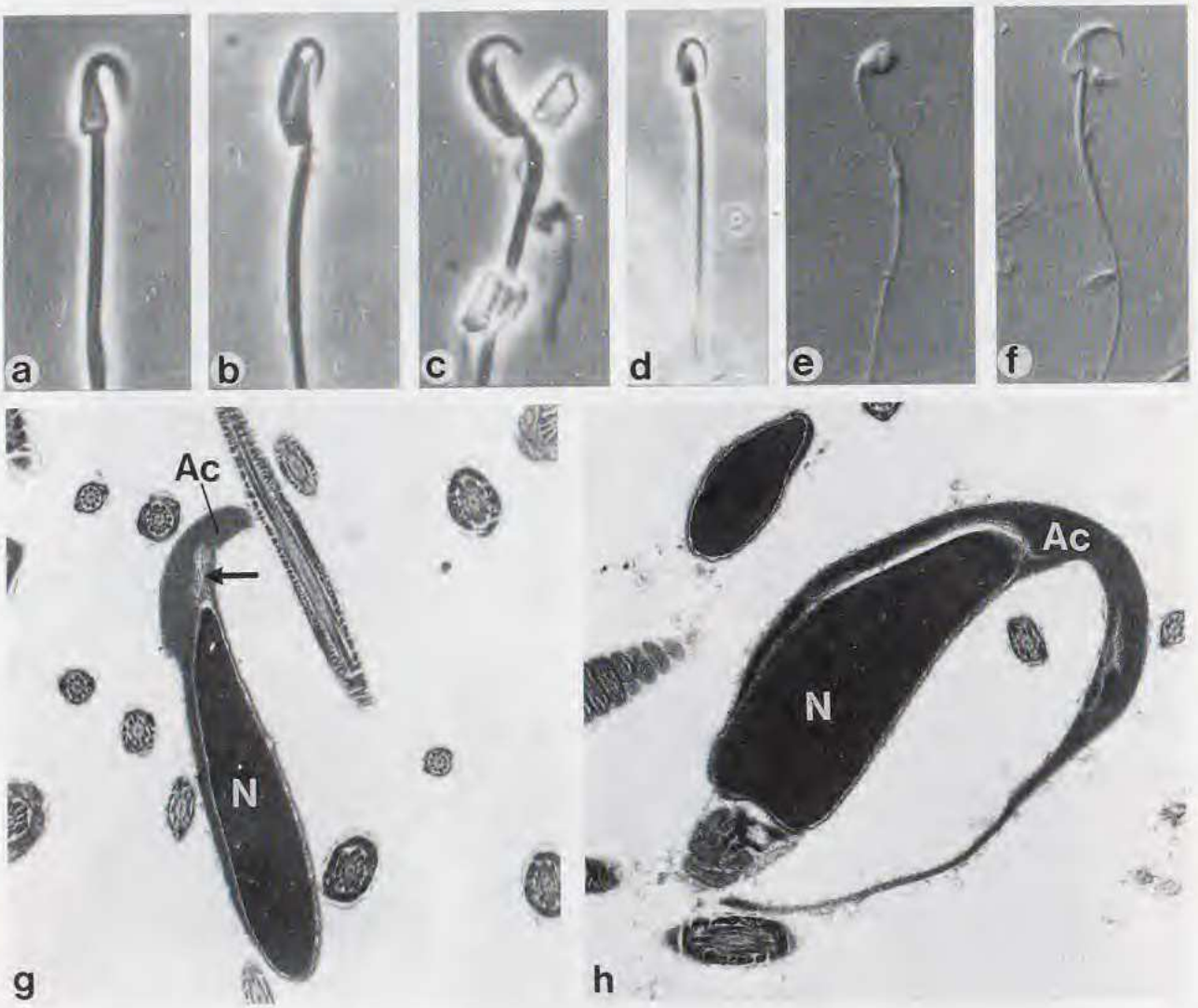


FIG. 4. — Sperm from dendromurine and otomyine species, light microscopy. **a:** *Prionomys batesi*; **b:** *Dendromus mystacalis*; **c:** *Dendromus mesomelas*; **d:** *Steatomys parvus*; **e:** *Deomys ferrugineus*; **f:** *Otomys irroratus*. **g, h:** *Malacothrix typica*, TEM of sperm head. x 700; b, x 1 600; c, x 1 600; d, x 700; e, x 1 100; f, x 600; g, x 9 100; h, x 11 000. a-d, phase contrast; e, f, Nomarski optics.

DISCUSSION

Most eutherians have a spatulate sperm head nucleus that is capped by an acrosome of variable size, but in most murine rodents the sperm head is falciform with the tail attached to the lower concave surface [6-10, 20]; there is a very large and elaborate apical perforatorium which passes back over the anterior nuclear region as three prongs [25, 33]. The sperm head of the golden hamster (a member of the Cricetinae) is also falciform, but its ultrastructure is very different with the acrosome occurring as a large cap over the dorsal and upper lateral nuclear surface, a bilaterally flattened apical hook, and the sperm tail attached to the head midbasally [19, 29, 41]. The ultrastructure of the sperm head of *Mystromys alba* in general resembles that of the golden hamster except that the sperm head is a little narrower and there is a much larger apical acrosomal segment.

In *Saccostomus campestris* the ultrastructural characteristics of the acrosome, perforatorium, and site of attachment of the connecting piece of the sperm tail are somewhat similar to those of the golden hamster and *Mystromys alba* although the perforatorium and acrosome are less extensive apically. This similar internal structural organisation in these three species, two of which come from subfamilies outside the dendromurine-cricetomyine-otomyine-murine clade, suggests very strongly that it represents the ancestral type for this group.

Amongst the dendromurines *Deomys* has sometimes been separated from the others and even placed within its own subfamily [17]. The present data on sperm morphology support the view that *Deomys* is highly divergent, whereas the ultrastructural characteristics of sperm head and site of tail attachment of *Malacothrix typica* are similar to those of *Saccostomus campestris*. The light microscopical observations suggest that *S. parvus* and *P. batesi* sperm are also similar. The ultrastructure of *M. typica* sperm does not resemble that of most of the murines [8, 9, 25] which probably have a derived sperm type (see below). Thus there is no support for close affinity of these groups from these data.

The position of the gerbils (subfamily Gerbillinae) has changed over the years. SIMPSON [37] placed this subfamily within the Cricetidae, but subsequent biochemical data suggested that the Gerbillinae could be the sister-group of the Murinae [13]. The present study indicates that the spermatozoon structure of both *Tatera leucogaster* and *Gerbillurus paeba* is highly derived and quite unlike that of any other genus outside the Gerbillinae; no phylogenetic inferences can thus be drawn.

Over the last 10 years it has become apparent that *Acomys*, previously thought to be a typical murine, may not in fact be part of this large African murid group [36, 40]. It has subsequently been suggested that *Acomys*, *Uranomys* and *Lophuromys* form a clade that is either an early offshoot of the Murinae or closer to the gerbils [13, 15, 16]. The ultrastructure of the sperm acrosome and perforatorium of both *Acomys* and *Uranomys* is more similar to that of *Saccostomus campestris* and the golden hamster than to that of typical murines. However, unlike sperm of *S. campestris*, dendromurines, and cricetines, the sperm tail attaches to the lower concave surface of the sperm head similar to that of the falciform sperm of murine rodents [5]. This trait is thus shared between most members of the Murinae and *Acomys-Uranomys* to the exclusion of the other groups except the Otomyinae [2]. These data support the view that the *Acomys-Uranomys* (and perhaps *Lophuromys*, which has a highly derived sperm type [6]) clade may have branched off from the base of the murine-otomyine radiation [4, 16, 30]. Furthermore, the present study extends the recent observations on the sperm of *Otomys* [2, 5] in confirming that the head is falciform and showing that the structure of the acrosome and perforatorium, as well as the site of attachment of the sperm tail, is identical to that of murines [1, 3, 5-10, 25]. These ultrastructural characteristics of the sperm head do not occur in species from any other subfamily nor do they occur in *Acomys* and *Uranomys*. They are thus likely to be a synapomorphic character shared by most members of the Otomyinae and Murinae to the exclusion of *Acomys* and *Uranomys* and other subfamilies. The findings thus support the previous

suggestion [5] that these two subfamilies are sister-groups and that, in spite of a recent claim to the contrary [35], the falciform sperm head is the ancestral condition within the Murinae.

The following tentative phylogenetic conclusions can be drawn from this study:

1. *Saccostomus* is not part of the Murinae.
2. Within the Dendromurinae, *Deomys* is highly derived and may not be close to the other members of this group.
3. The Dendromurinae is not particularly close to the Murinae.
4. The Otomyinae is the sister-group of the Murinae.
5. The *Acomys-Uranomys* lineage diverged from the base of the Murine-Otomyine clade.

Clearly further insight into the phylogenetic relationships of African muroids could be achieved by carrying out electron microscopy on spermatozoa of more species when, or if, they become available; sperm from more genera of dendromurines and gerbillines need to be investigated. In this study there was no material available from any of the Malagasy rats and mice (subfamily Nesomyinae), maned rats (subfamily Lophiomyinae), or rock and climbing swamp mice (subfamily Petromyscinae). Spermatozoal morphology of these species needs to be investigated before a full appreciation of its significance to the understanding of the evolution of the rats and mice of Africa can be ascertained.

ACKNOWLEDGEMENTS

I thank Prof. M. ISAACSON and Ms. P. HAWKINS of SAIMR, Prof. M. PERRIN of Department of Zoology, University of Natal, Pietermaritzburg, and Mr. G. BRONNER of the Transvaal Museum, Pretoria for material. Ms. P. JENKINS of the Natural History Museum, London, kindly allowed me access to the dendromurines. I should like to thank Mr. C. LEIGH and Mr. R. MURPHY of the University of Adelaide for technical assistance, and Esther BREED for typing the manuscript. Material for this study was obtained whilst the author was on study leave from The University of Adelaide.

REFERENCES

1. AUSTIN, C. R. & BISHOP, M. W. H., 1958. — Some features of the acrosome and perforatorium in mammalian spermatozoa. *Proceedings of the Royal Society of London B.*, **149**: 234-240.
2. BERNARD, R. J. F., HODGSON, A. N. & CAMPBELL, G. K., 1991. — Sperm structure and taxonomic affinities of five African rodents of the subfamily Otomyinae. *South African Journal of Science*, **87**: 503-506.
3. BISHOP, M. W. H. & AUSTIN, C. R., 1957. — Mammalian spermatozoa. *Endeavour*, **16**: 137-150.
4. BONHOMME, F., ISKANDER, D., THALER, L. & PETTER, F., 1985. — Electromorphs and phylogeny in muroid rodents. *In*: W. P LUCKETT & J. L. HARTENBERGER, *Evolutionary Relationships among Rodents*. New York, Plenum Press: 671-683.
5. BREED, W. G., 1991. — Variation in sperm head morphology of the Murinae (Family Muridae); some taxonomic implications. *In*: B. BACCETTI, *Comparative Spermatology: 20 Years After*. New York, Raven Press: 937-939.
6. BREED, W. G., 1995. — Spermatozoa of African murid rodents: structural diversity and evolution of form. *Journal of Zoology, London*, in press.
7. BREED, W. G. & SARAFIS, V., 1979. — On the phylogenetic significance of spermatozoal morphology and male reproductive tract anatomy in Australian rodents. *Transactions of the Royal Society of South Australia*, **103**: 127-139.
8. BREED, W. G. & YONG, H. S., 1986. — Sperm morphology of murid rodents from Malaysia and its possible phylogenetic significance. *American Museum Novitates*, **2856**: 1-12.
9. BREED, W. G. & LEIGH, C. M., 1991. — Distribution of filamentous actin in and around spermatids and in spermatozoa of Australian Conilurine rodents. *Molecular Reproduction and Development*, **30**: 369-384.
10. BREED, W. G. & MUSSER, G. G., 1991. — Sulawesi and Philippine rodents (Muridae); a survey of spermatozoal morphology and its significance for phylogenetic inference. *American Museum Novitates*, **3003**: 1-15.
11. BROWNELL, E., 1983. — DNA/DNA hybridisation studies of muroid rodents: symmetry and rates of molecular evolution. *Evolution*, **37**: 1034-1051.

12. CARLETON, M. D. & MUSSER, G. G., 1984. — Muroid rodents. *In*: S. ANDERSON & J. KNOX-JONES Jr., *Orders and Families of Recent Mammals of the World*. New York, John Wiley & Sons: 289-380.

13. CATZEFLIS, F. M., DICKERMAN, A. W., MICHAUX, J. & KIRSCH, J. A. W., 1993. — DNA hybridisation and rodent phylogeny. *In*: F. S. SZALAY, M. J. NOVACEK & M. C. MCKENNA, *Mammal Phylogeny - Placentals*. New York, Springer Verlag: 159-172.

14. CHALINE, J., MEIN, P. & PETTER, F., 1977. — Les grandes lignes d'une classification évolutive des Muroidea. *Mammalia*, **41**: 245-252.

15. CHEVRET, P., DENYS, C., JAEGER, Y.-J., MICHAUX, J. & CATZEFLIS, F. M., 1993. — Molecular evidence that the spiny mouse (*Acomys*) is more closely related to gerbils (Gerbillinae) than to true mice (Murinae). *Proceedings of the National Academy of Science*, **90**: 3433-3436.

16. DENYS, C., MICHAUX, J., PETTER, F., AGUILAR, J. P. & JAEGER, J. J., 1992. — Molar morphology as a clue to the phylogenetic relationship of *Acomys* to the Murinae. *Israel Journal of Zoology*, **38**: 253-262.

17. ELLERMAN, J. R., 1941. — *The Families and Genera of Living Rodents*. Vol II. London, British Museum (Natural History): 1-690.

18. FLYNN, L. J., JACOBS, L. L., & LINDSAY, E. H., 1985. — Problems in muroid phylogeny: relationship to other rodents and the origin of major groups. *In*: W. P. LUCKETT & J. C. HARTENBERGER, *Evolutionary Relationships amongst Rodents: a Multidisciplinary Analysis*. New York & London, Plenum Press: 589-616.

19. FRANKLIN, L. E., BARROS, C. & FUSSELL, E. N., 1970. — The acrosomal region and the acrosome reaction in sperm of the golden hamster. *Biology of Reproduction*, **3**: 180-200.

20. FRIEND, G. F., 1936. — The sperms of British Muridae. *Quarterly Journal of Microscopical Science*, **78**: 419-443.

21. GORDON, D. H. & WATSON, C. R. B., 1986. — Identification of cryptic species of rodents (*Mastomys, Aethomys, Saccostomus*) in the Kruger National Park. *South African Journal of Zoology*, **21**: 95-97.

22. HARDING, H. R., 1987. — Interrelationships of the families of Diprotodonta - a view based on spermatozoan ultrastructure. *In*: M. ARCHER, *Possums and Opossums: Studies in Evolution*. Sydney, Surrey Beatty & Sons and The Royal Society of New South Wales: 195-216.

23. HIRTH, H. F., 1960. — The spermatozoa of some North American bats and rodents. *Journal of Morphology*, **106**: 77-83.

24. HUGHES, R. L., 1965. — Comparative morphology of spermatozoa from five marsupial families. *Australian Journal of Zoology*, **13**: 533-543.

25. LALLI, M. & CLERMONT, Y., 1981. — Structural changes of the head components of the rat spermatid during spermiogenesis. *American Journal of Anatomy*, **160**: 419-434.

26. LAVOCAT, R., 1959. — Origine et affinitiés des rongeurs de la sous-famille des dendromurinés. *Comptes Rendus des Séances de l'Académie des Sciences, Paris*, **248**: 1375-1377.

27. LINZEY, A. V. & LAYNE, J. N., 1974. — Comparative morphology of spermatozoa of the rodent genus *Peromyscus* (Muridae). *American Museum Novitates*, **2532**: 1-20.

28. MISONNE, X., 1969. — African and Indo-Australian Muridae. Evolutionary trends. *Annales (Série 8) Musée Royal de l'Afrique Centrale, Tervuren, Zoologie*, **172**: 1-219.

29. OLSON, F. E. & WINFREY, V. P., 1985. — Substructure of a cytoskeletal complex associated with the hamster sperm acrosome. *Journal of Ultrastructural Research*, **92**: 167-179.

30. PASCALE, E., VALLE, E. & FORANO, A. V., 1990. — Amplification of an ancestral mammalian L1 family of long interspersed repeated DNA occurred just before the murine radiation. *Proceedings of the National Academy of Science*, **87**: 9481-9485.

31. PETTER, F., 1966a. — Affinités des genres *Beamys*, *Saccostomus* et *Cricetomys* (Rongeurs, Cricetomyinae). *Annales (Série 8) Musée Royal de l'Afrique Centrale, Zoologie*, **144**: 13-25.

32. PETTER, F., 1966b. — L'origine des murides. plan cricetin et plans murins. *Mammalia*, **30**: 205-225.

33. PIKO, L., 1969. — Gamete structure and sperm entry in mammals. *In*: C. B. METZ & A. MONROY, *Fertilization*. New York & London, Academic Press: 325-403.

34. REIG, O. A., 1980. — A new fossil genus of South American cricetid rodents allied to *Wiedomys*, with an assessment of Sigmodondinae. *Journal of Zoology, London*, **192**: 257-281.

35. ROLDEN, E. R. S., GOMENDIO, M. & VITULLO, A. D., 1992. — The evolution of eutherian spermatozoa and underlying selective forces: female selection and sperm competition. *Biological Reviews*, **647**: 551-593.

36. SARICH, V. M., 1985. — Rodent macroevolution systematics. *In*: W. P. LUCKETT & J. C. HARTENBERGER, *Evolutionary Relationships amongst Rodents: a Multidisciplinary Analysis*. New York & London, Plenum Press: 423-452.

37. SIMPSON, G. G., 1945. — The principles of classification and a classification of mammals. *Bulletin of the American Museum of Natural History* 85 xvi. 1-350.

38. TEMPLE-SMITH, P. D., 1987. — Sperm structure and marsupial phylogeny. *In*: M. ARCHER, *Possums and Opossums: Studies in Evolution*. Sydney, Surrey Beatty & Sons and The Royal Society of New South Wales: 171-193.

39. VISSER, D. S. & ROBINSON, T. J., 1987. — Systematic implications of spermatozoa and bacular morphology for the South African *Aethomys*. *Mammalia*, **51**: 447-454.

40. WILSON, A. C., OCHMAN, H. & PRAGER, E. M., 1987. — Molecular time scales for evolution. *Trends in Genetics*, **3**: 241-247.

41. YANAGIMACHI, R. & NODA, Y. D., 1970. — Fine structure of the hamster sperm head. *American Journal of Anatomy*, **128**: 367-388.